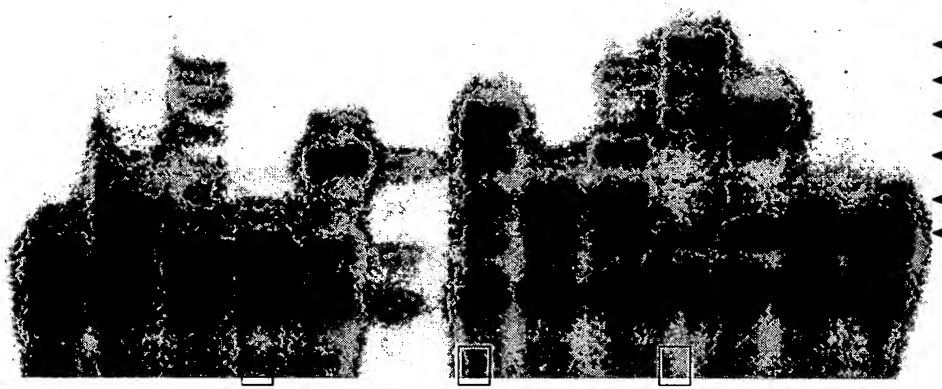
Incorporated (5' to 3')

GATC AGAAAG



5 base extension
4 base extension
3 base extension
2 base extension
1 base extension
19 base "TOP"

dNTP - G A A* G A* A* G A A

LANE 1 2 3 4 5 6 7 8 9 10 11 12 13

*implies presence of an ANS tag attached via the dNTP γ-phosphate

FIG. 1

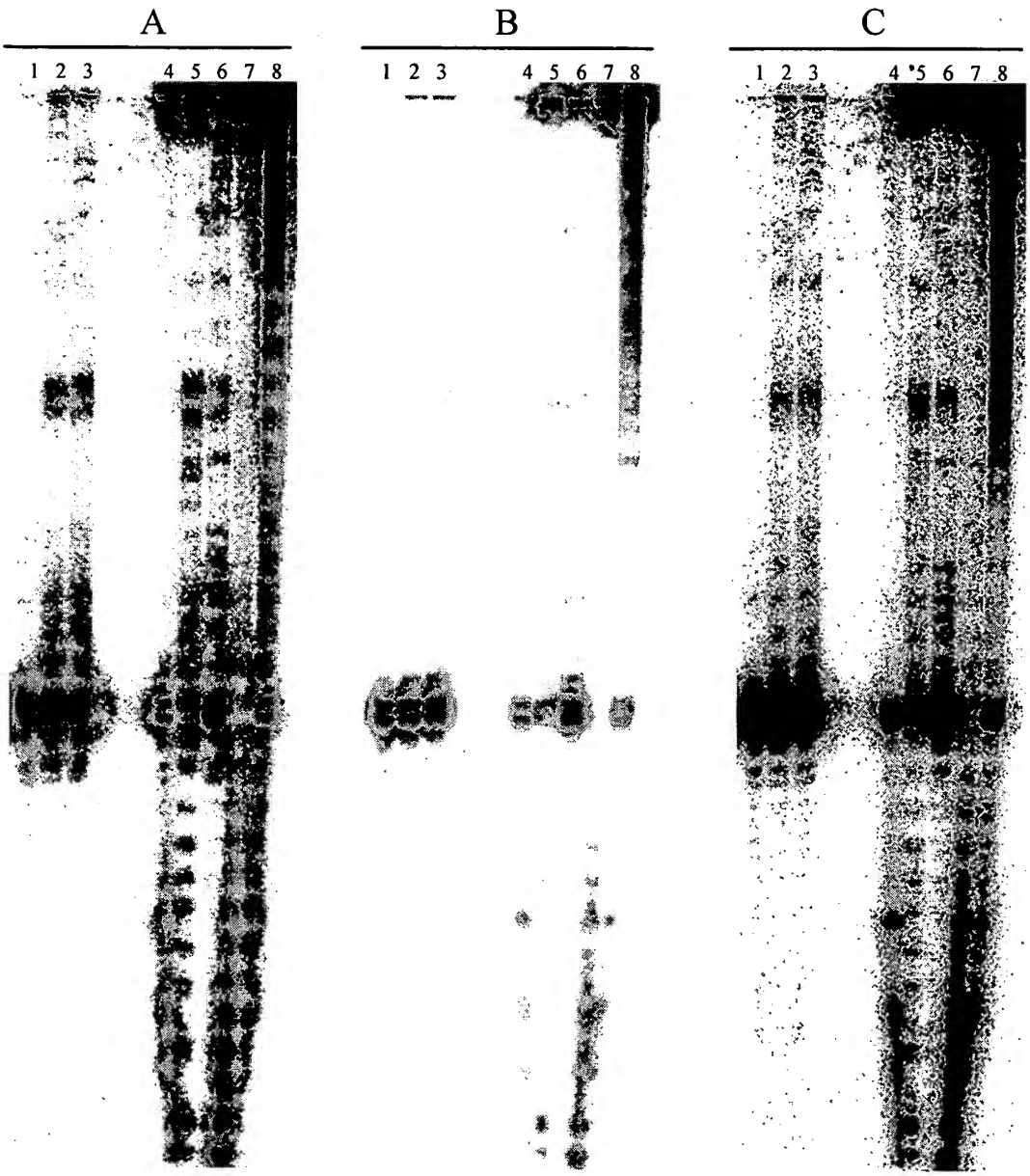


FIG. 2

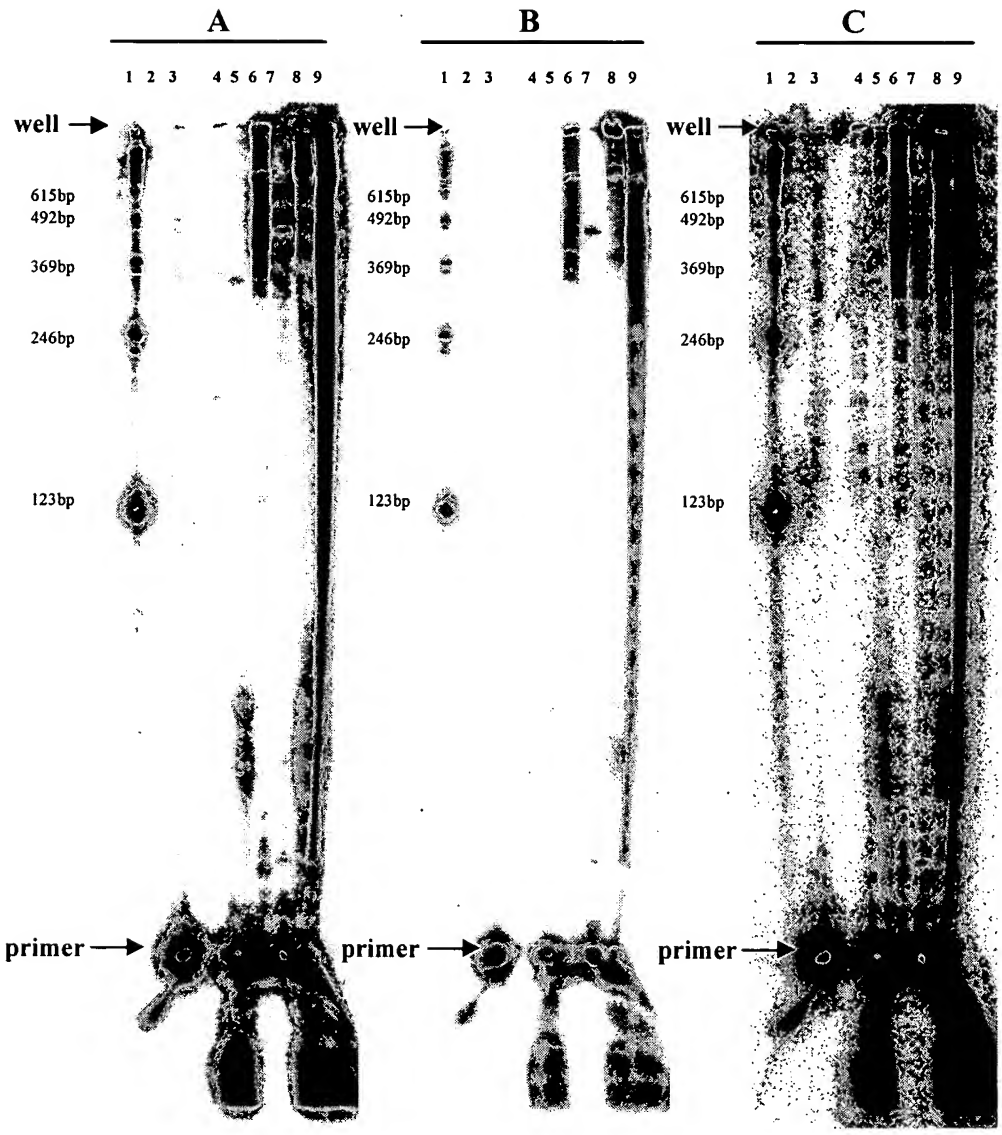
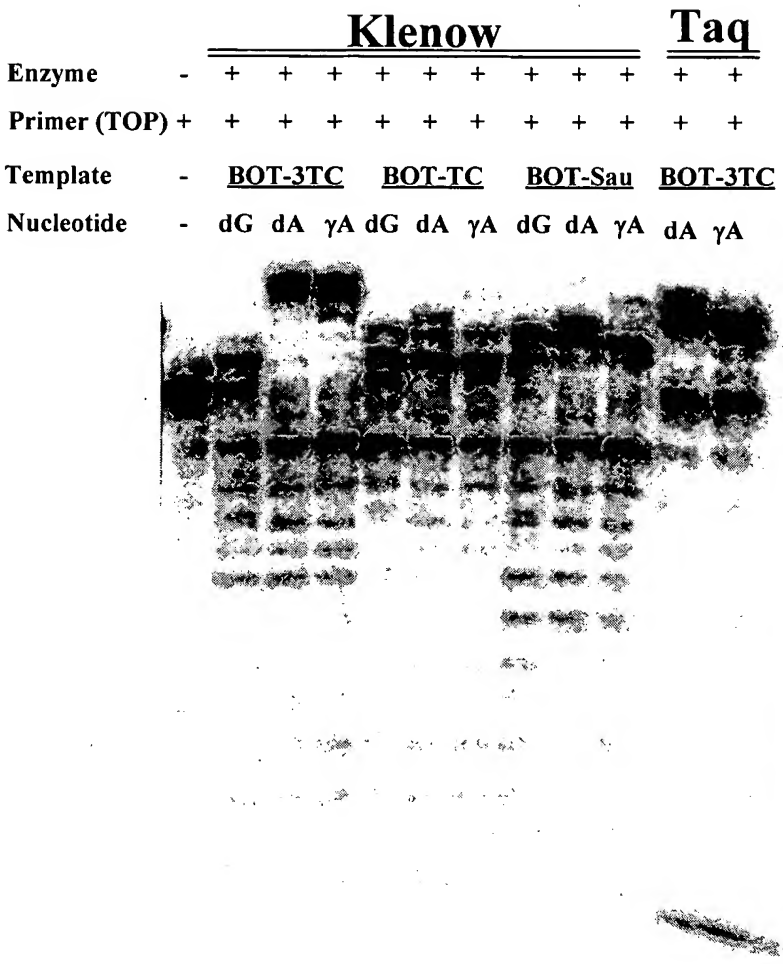
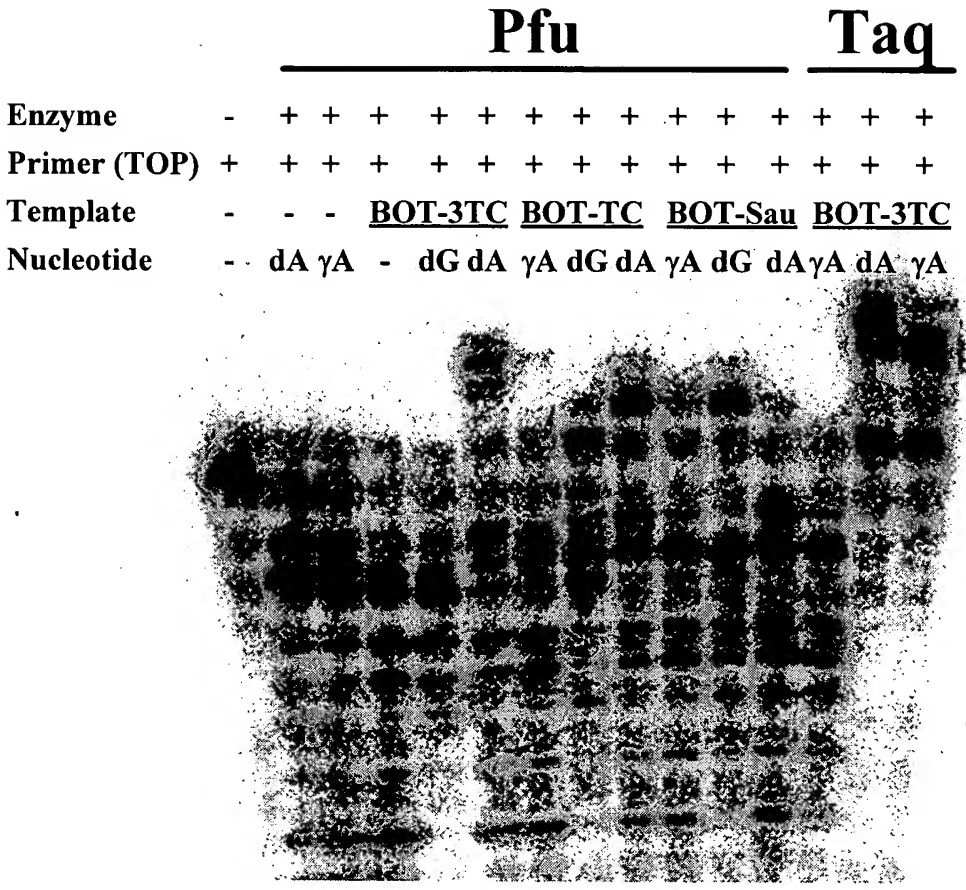


FIG. 3



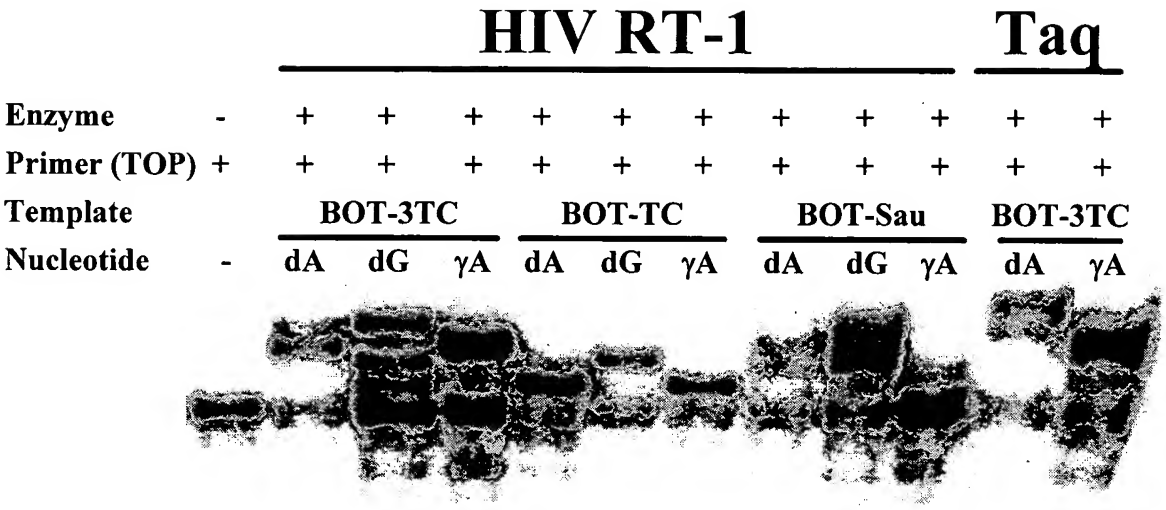
γ implies the presence of an ANS-tag attached at the dNTP γ-phosphate

FIG. 4



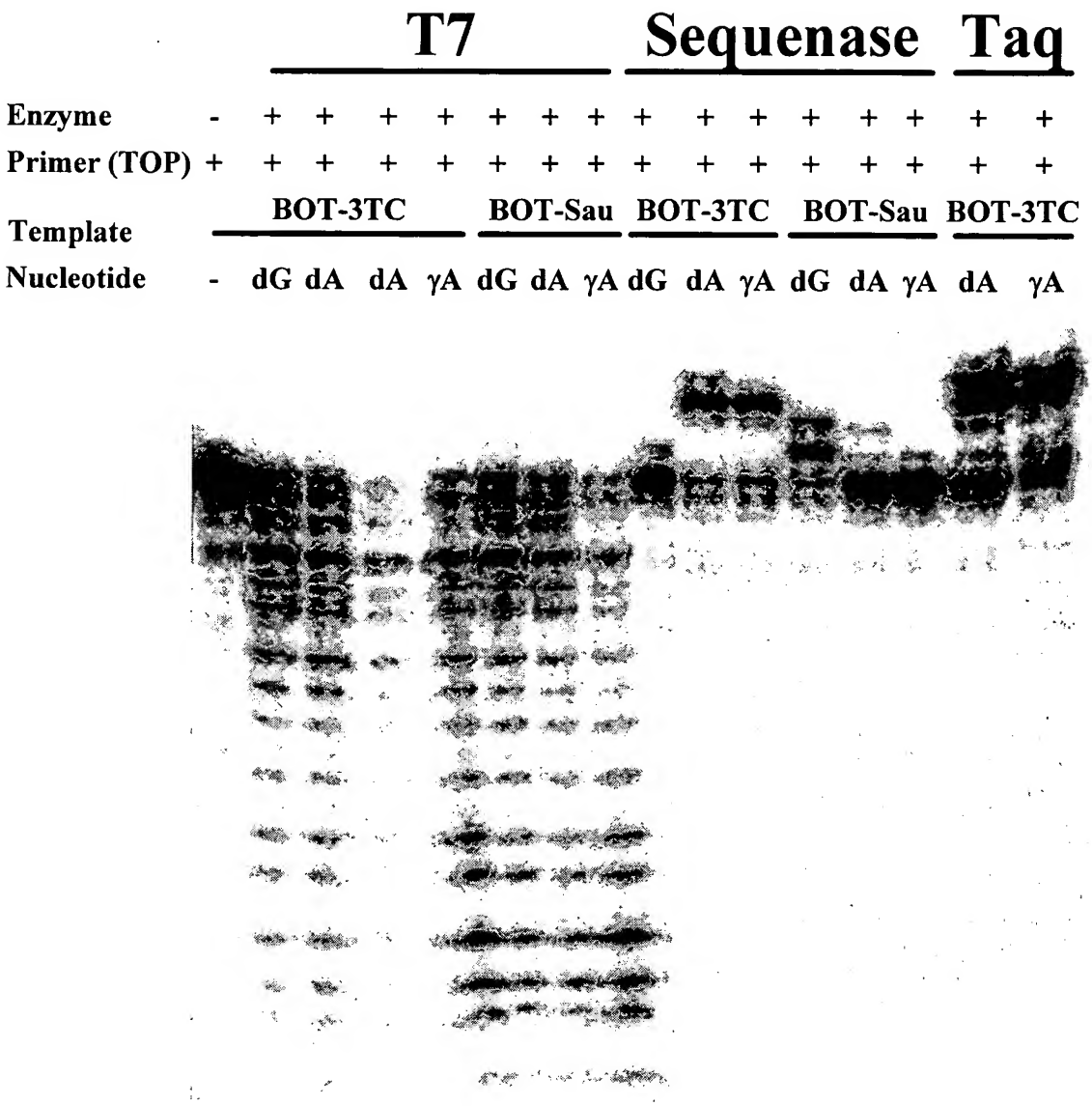
γ implies presence of an ANS-tag attached via the dNTP γ–phosphate

FIG. 5



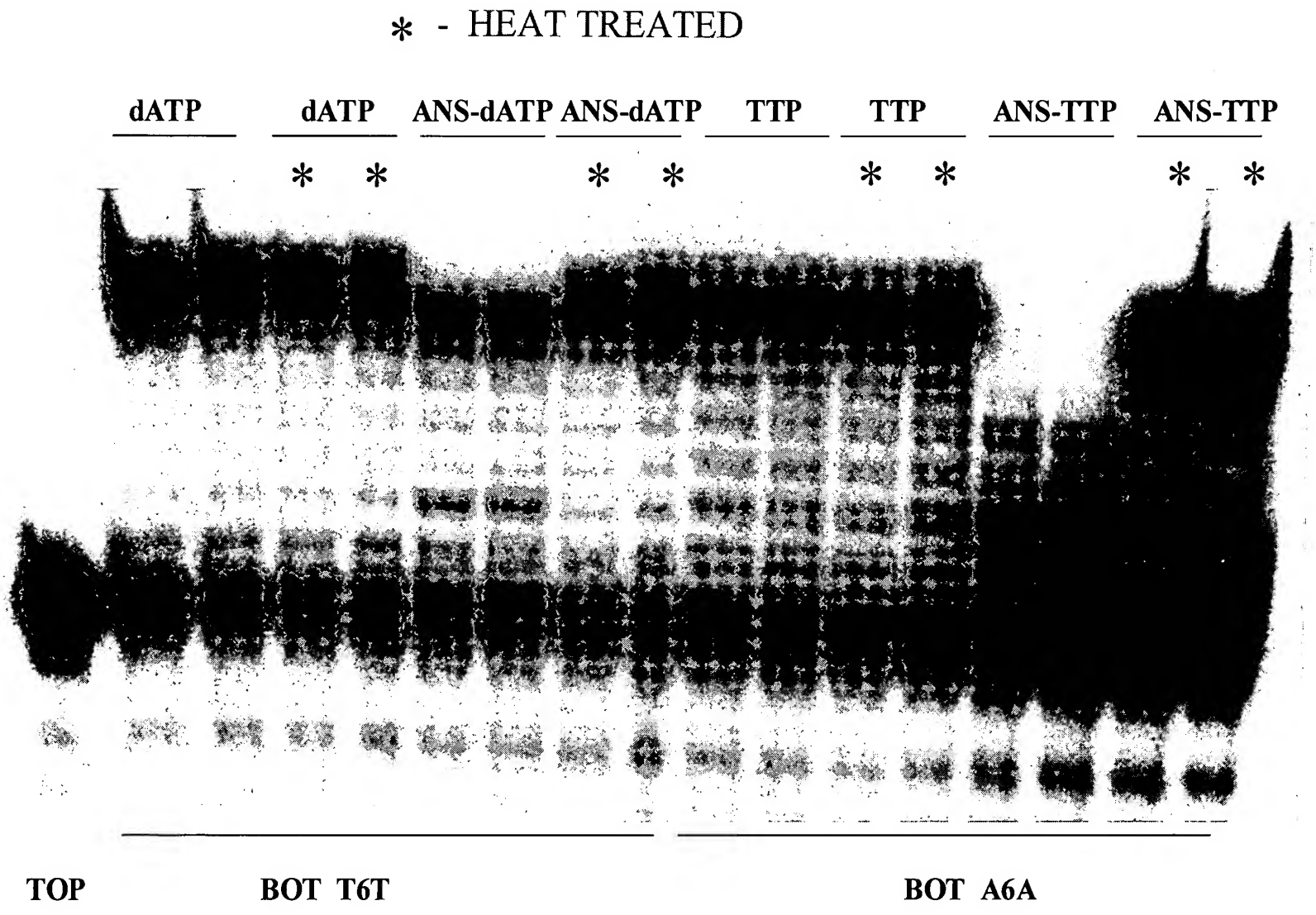
γ implies presence of an ANS-tag attached via the dNTP γ–phosphate

FIG. 6



γ implies presence of an ANS-tag attached via the dNTP γ–phosphate

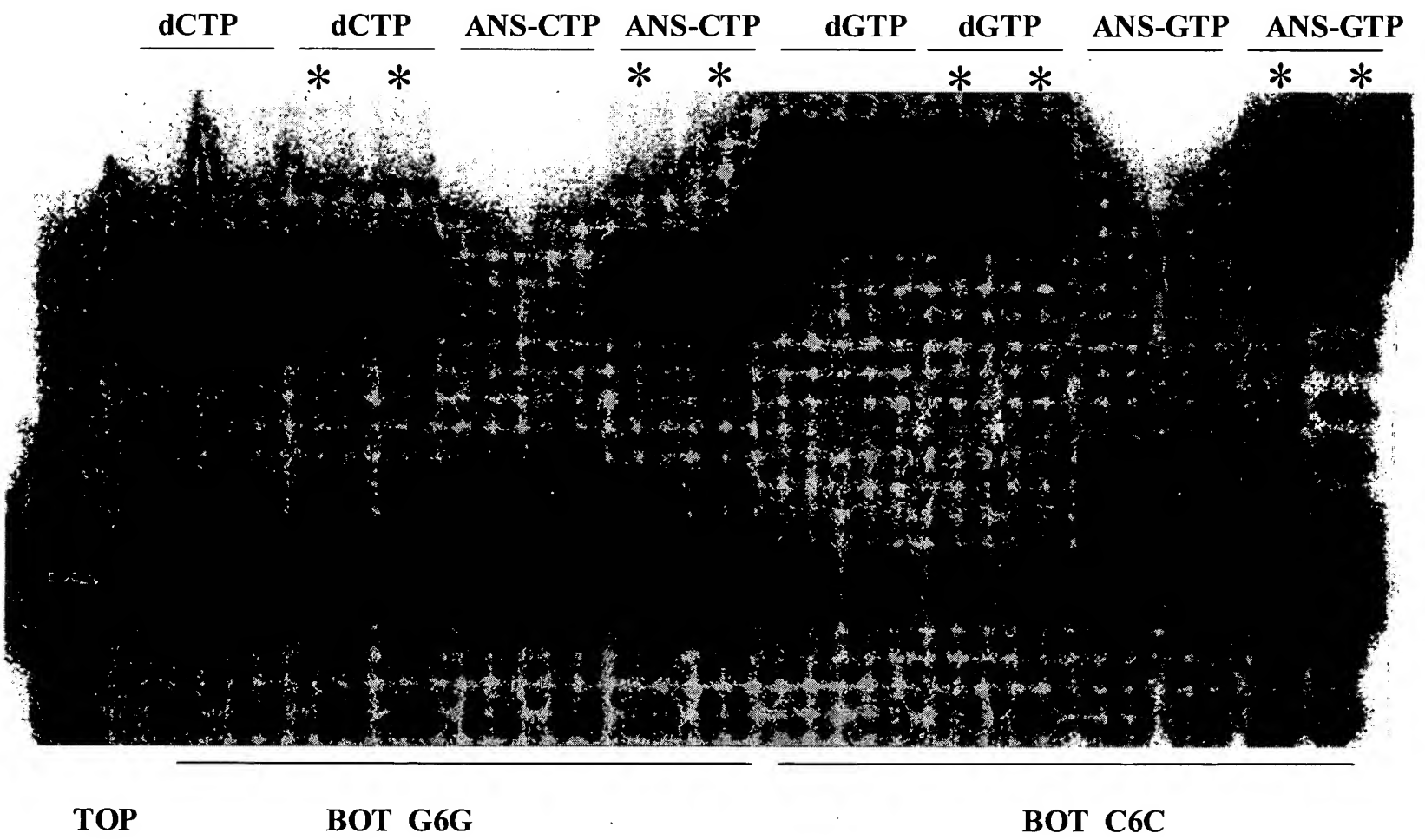
FIG. 7



10μM each dNTP; *Taq DNA Polymerase; extension 30' @ 37°C*

FIG. 8

* - HEAT TREATED



10μM each dNTP; *Taq DNA Polymerase; extension 30' @ 37°C*

FIG. 9

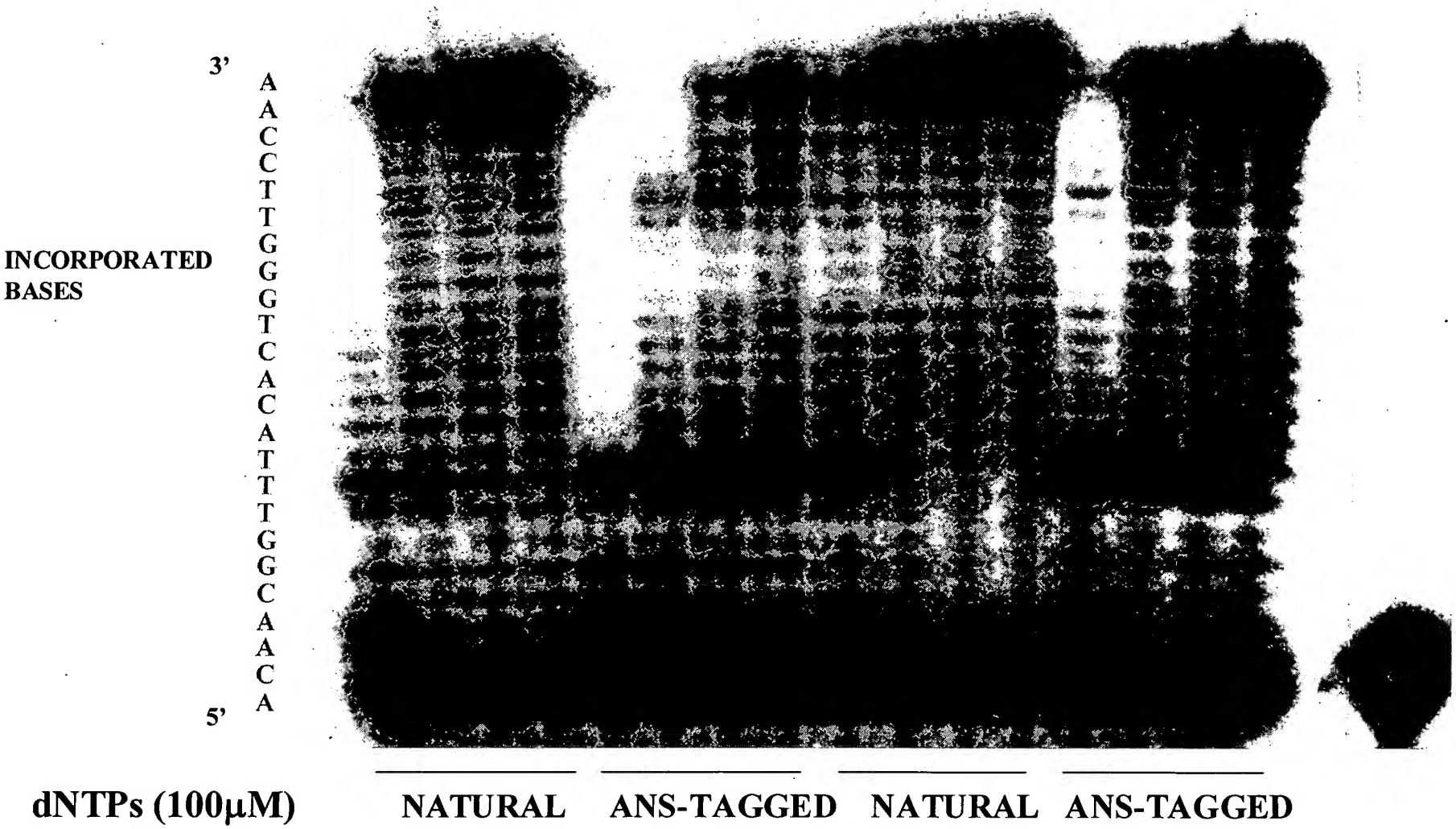

EXTENSION TIME
1 minute
10 minutes
TEMPERATURE
10° 20° 30° 40° 10° 20° 30° 40° 10° 20° 30° 40° 10° 20° 30° 40°
3'
AACCTTGGGTCACATTTGGCAACA
5'
INCORPORATED BASES
dNTPs (100μM)
NATURAL
ANS-TAGGED
NATURAL
ANS-TAGGED

FIG. 10

Primer sequence: 5' GGTACTAAGCGGCCGCATG 3'

Template sequence: 3' CCATGATTCGCCGGCGTACTGTTGCCAAATGTGACCCAAGGTT 5'

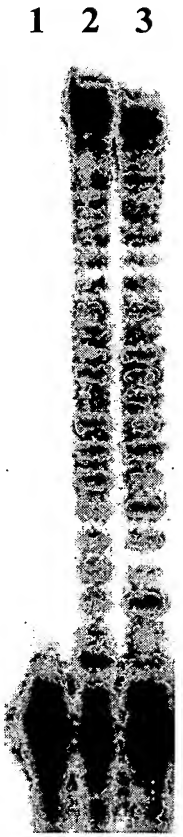


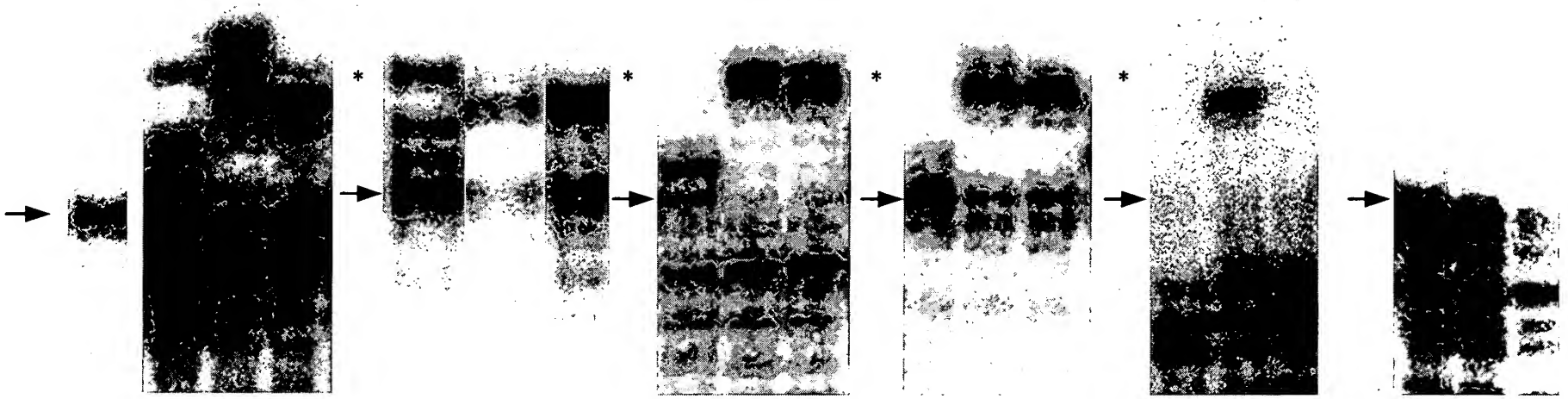
FIG. 11

Primer sequence: 5' GGTACTAAGCGGCCGCATG 3'

Template sequence: 3' CCATGATTCGCCGGCGTACTTTC5'

POLYMERASE

Taq **HIV RT-1** **Klenow** **Sequenase** ***Pfu*** **T7**



Different Polymerases React Differently to the ANS-g-modified Nucleotides: primer extension reactions were performed to determine the ability of various polymerases to incorporate -tagged dNTPs during DNA polymerization.Control reactions contained natural dNTPs to monitor for template-directed nucleotide incorporation as well as for misincorporation. The reactions were performed in the appropriate buffer and contained the specified polymerase, primer/template duplex (radiolabeled 'TOP'primer annealed to 'BOT-3TC'template), and only the indicated dNTP. The reactions were carried out at room temperature or at 37C for 30 minutes and were stopped by the addition of 0.5mM EDTA. The volume of the reaction was then reduced to approximately 2-4l, loading dye was added and the polymerization products were electrophoresed through a 20% denaturing polyacrylamide gel.Arrows indicate the position of the free labeled 'TOP'. Asterisks indicate 3-base extension.

FIG. 12

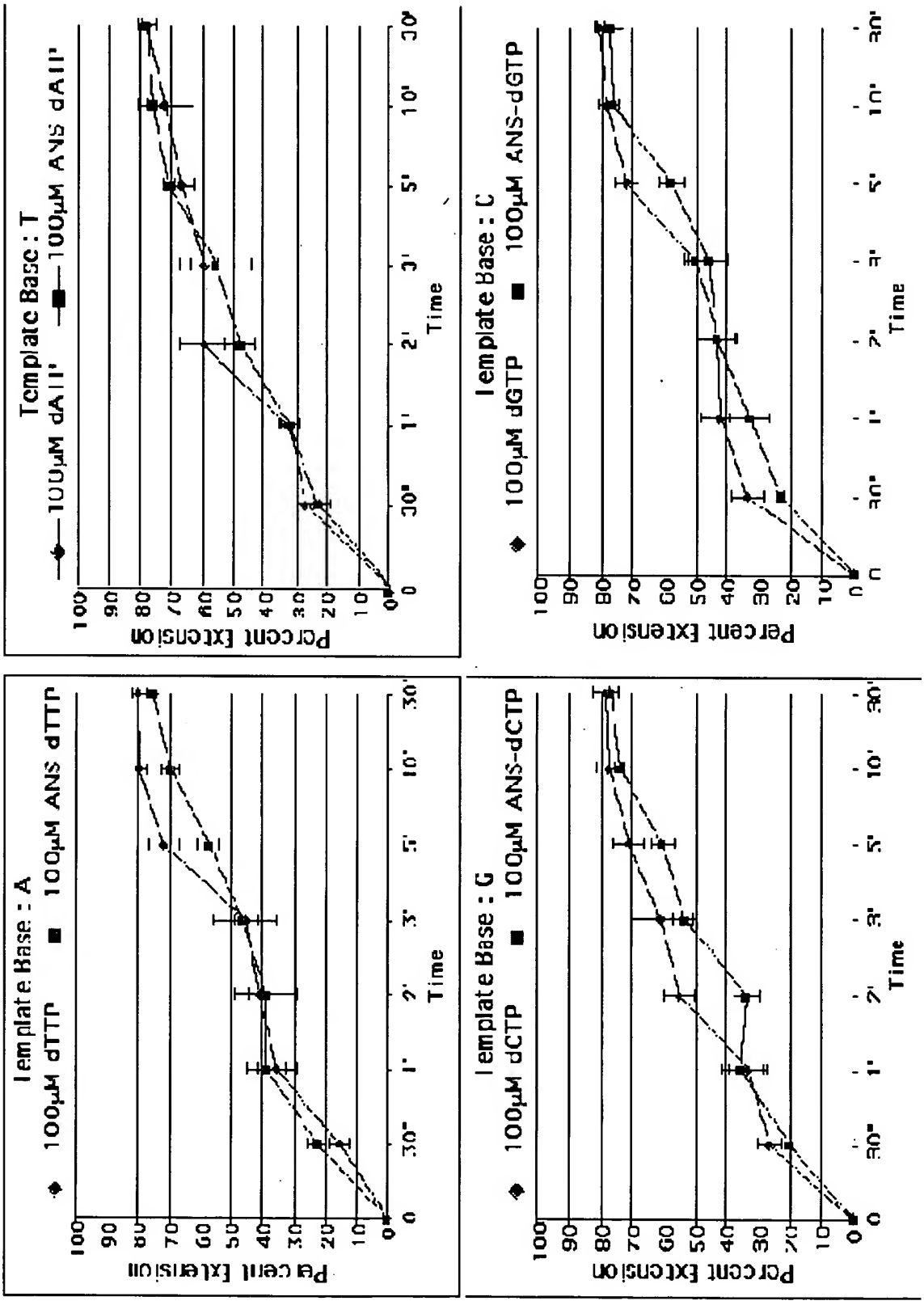

Template Base : T
100μM dATP
100μM ANS dATP
Template Base : A
100μM dTTP
100μM ANS dTTP
Template Base : C
100μM dGTP
100μM ANS-dGTP
Template Base : G
100μM dCTP
100μM ANS-dCTP
Percent Extension
Time

FIG. 13

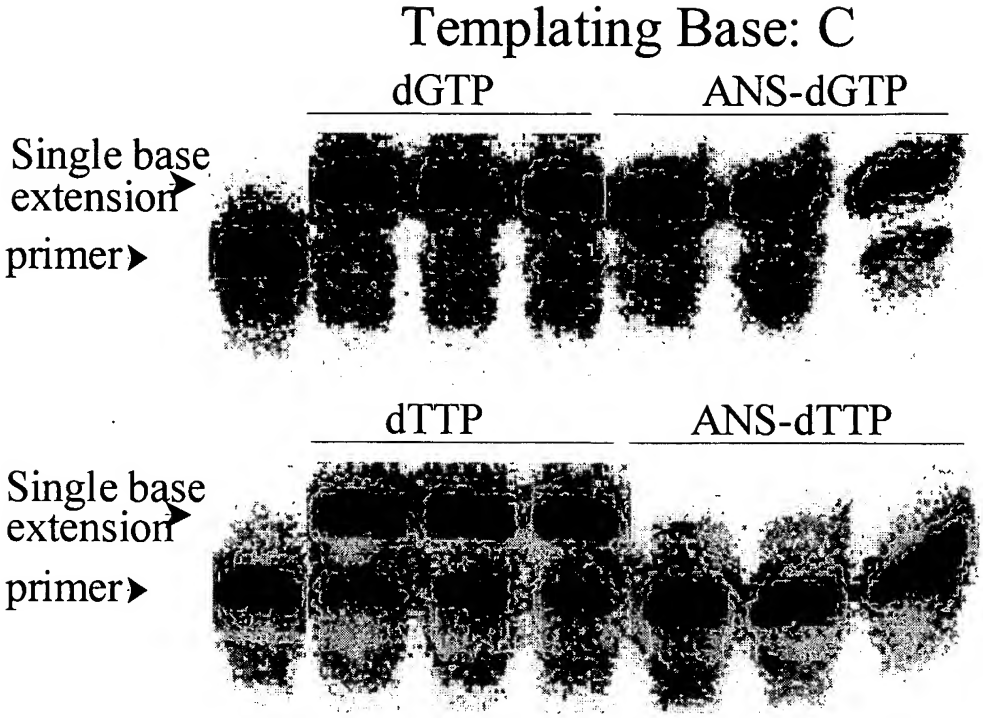


FIG. 14 TOP

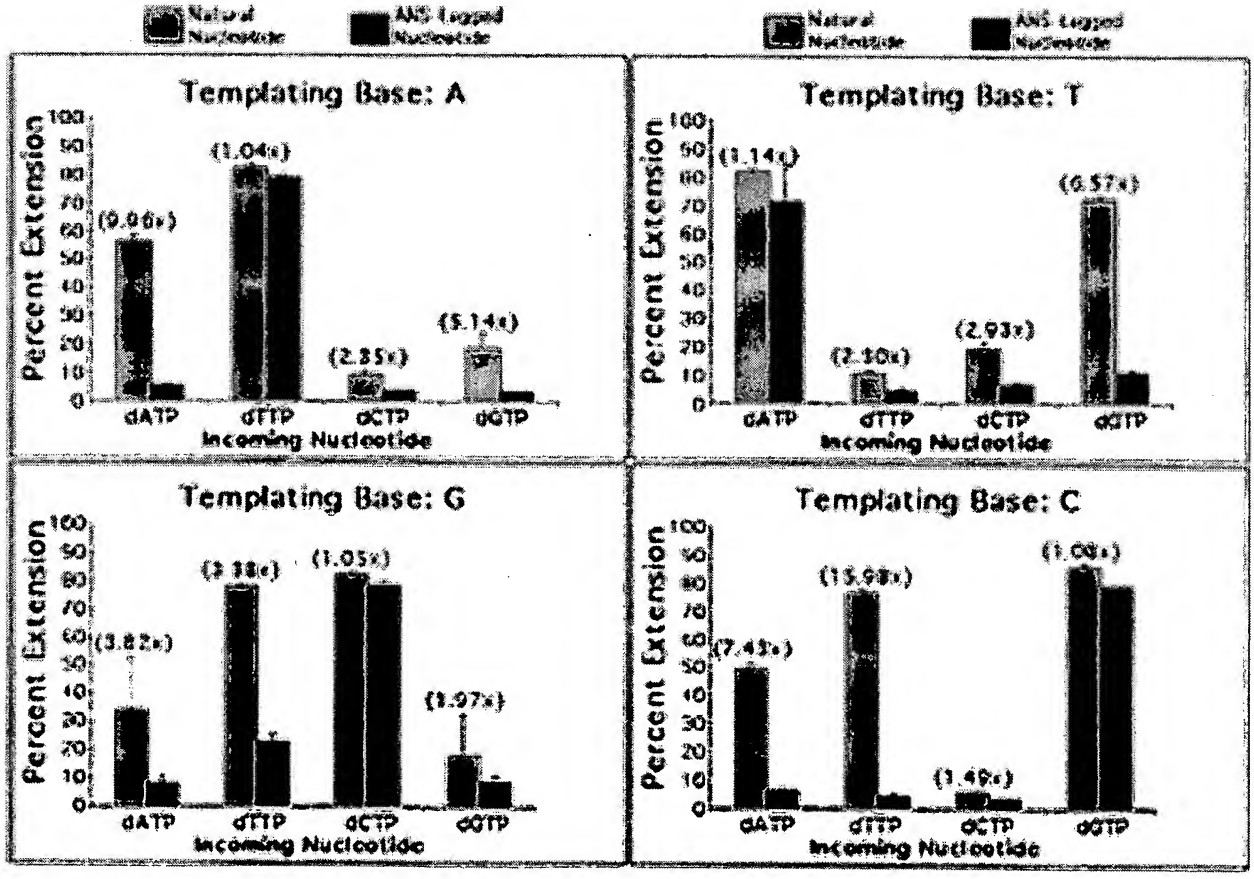


FIG. 14 BOTTOM